

DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

Version
January 1997

Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH

and

COLLABORATING INVESTIGATORS

ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 ANTIGENS

Abbott HIVAG-1 Polyclonal Culture Supernatant Assay

I. PRINCIPLE

The Abbott HIVAG-1 Polyclonal Assay is an enzyme immunoassay (EIA) developed for the detection of HIV-1 p24 antigen. It may be used to detect the presence of p24 antigen in the supernatant of a HIV-1 culture. The Abbott HIVAG-1 Polyclonal Assay is a “sandwich” solid phase immunoassay that uses polystyrene beads coated with a human polyclonal antibody (Ab) to HIV-1 p24. If present, the viral p24 antigen (Ag) binds to the antibody coated bead. Following a wash step, rabbit antibody to HIV-1 is then incubated with the beads and binds to the HIV-1 Ag. Goat antibody to rabbit IgG conjugated with horseradish peroxidase (anti-Rabbit IgG: HRPO) is incubated with the beads and binds to rabbit antibody. Next o-Phenylenediamine (OPD) Solution containing hydrogen peroxide is added to the bead, and after incubation, a yellow-orange color develops in proportion to the amount of HIV-1 antigen bound to the bead. The quantity of HIV-1 antigen in a specimen is determined by comparing its absorbance with that of a known HIV-1 p24 antigen standard curve.

II. SPECIMEN REQUIREMENTS

Aliquots of culture supernatant should be collected and frozen at -20°C until tested. Heat-inactivated specimens or specimens with obvious microbial contamination are unacceptable. Avoid subjecting specimens to repeated freeze thaw cycles.

Bring all samples to room temperature ($15\text{--}30^{\circ}\text{C}$) prior to assay.

III. REAGENTS

- A. The following reagents are included in the Abbott HIVAG-1 Polyclonal Kit. Kit reagents may be used at room temperature or cold unless otherwise stated in reagent preparation.
1. HIV-1 (Human) Polyclonal Antibody-coated Beads. Store at $2\text{--}8^{\circ}\text{C}$. Note manufacturer's outdate. Replace desiccant after use and cap tightly for storage.
 2. Antibody to HIV-1 (Rabbit). Store at $2\text{--}8^{\circ}\text{C}$. Note manufacturer's outdate.
 3. Anti Rabbit IgG Conjugate (Goat). Store at $2\text{--}8^{\circ}\text{C}$. Note manufacturer's outdate.
 4. Diluent for OPD tablets. Store at $2\text{--}8^{\circ}\text{C}$. Note manufacturer's outdate.

5. OPD Tablets (o-Phenylenediamine-2HCL). Store at 2-30⁰C. Note manufacturer's outdate. Prepare OPD Substrate Solution as follow:
 - a. Bring OPD tablets and diluent to room temperature before use. Do not open tablet bottle until it reaches room temperature.
 - b. Within 5-60 minutes of use, prepare sufficient OPD Substrate Solution by dissolving the OPD Tablets in the OPD Diluent. See chart below.

No. Of Beads	Tablets	Diluent (mL)
13	1	5
28	2	10
43	3	15
58	4	20
73	5	25
88	6	30
103	7	35

- c. Just prior to dispensing, swirl the container gently to obtain a homogenous solution.

Note: Do not use an OPD tablet that is broken. Transfer tablets with non-metallic forceps into a metallic free bottle. OPD Substrate solution must be used within 60 minutes. OPD Substrate must not be exposed to strong light. Do not cap tightly while dissolving

6. Specimen Diluent containing Triton X-100. Store at 2-8⁰C. Note manufacturer's outdate.
7. Stop Solution (1N H₂SO₄). Store at 2-30⁰C. Note manufacturer's outdate.
8. Negative Control. Store at 2-8⁰C. Note manufacturer's outdate.

B. Reagents required but not provided:

1. 5% Hypochlorite solution (household bleach) diluted 1/100 or appropriate disinfectant.
2. Deionized or distilled water.

C. Standards and controls for the assay provided by the Virology Quality Assurance Laboratory (VQA):

1. VQA 400 pg/mL Standard. Store one vial designated as “Working Standard” at 2-8°C for up to one month. Other vials should be stored at -70°C or lower. Dilutions for standard curve should be made fresh for each assay as follows:
 - a. Make four 5-fold dilutions of the VQA 400 pg/mL Standard using VQA Diluent for dilutions.
 - b. Pipette 500 l of VQA Diluent into four tubes. Pipette 500 l of VQA 400 pg/mL Standard into the first tube and mix. Changing tips, pipette 500 l of the first dilution into the second tube of diluent and mix.
 - c. Continue until four dilutions are made.
2. VQA QC (Media Quality Control). A set of three concentrations. One vial of each concentration may be stored at 2-8°C for up to one month. Other vials should be stored at -70°C or lower.
3. VQA Diluent. One bottle of diluent may be stored at 2-8°C for up to one month. Other bottles should be stored at -70°C or lower.

IV. SUPPLIES AND EQUIPMENT

Lab coat

Gloves

Reaction Trays (Abbott)

Assay Tubes (Abbott)

Cover seals (Abbott)

Micropipet(s) capable of delivering 20, 50, 180 µL volumes

Precision pipettes, or similar equipment to deliver 200 µL, 300 µL, and 1 mL

Disposable pipette tips suitable for the above pipettes

Disposable serological pipettes

Disposable reagent reservoirs

Vortex mixer

Commander Dynamic Incubator (Abbott) or other incubator capable of 40 ± 2°C

Graduated cylinders and beakers

Washing device for washing beads such as Quickwash® or Pentawash® II with vacuum source and a double trap for retaining the aspirate, and capable of delivering a total rinse volume of 4-6 mL per well

Quantumatic™ or Quantum Analyzer™ or spectrophotometer able to read absorbance at 492 nm

Bead dispenser (Abbott)-optional

Non-metallic forceps

Metal free container for OPD Substrate Solution can be plastic or acid washed glassware

V. PROCEDURE

1. Create an EIA template in the virology data-management software (see software manual).
2. In an Abbott EIA reaction tray, dispense 20 μ L of Specimen Diluent to each well except the wells receiving the VQA standard dilutions and QC samples.
3. Dispense 20 μ L of Abbott negative control to each well except the wells receiving the VQA standard dilutions and QC samples.
4. Dispense 200 μ L uninfected donor culture supernatant into the first 3 wells of the reaction tray.
5. Transfer 200 μ L of the VQA standard dilutions, QC samples and patient specimens into the corresponding wells in the reaction tray according template.
6. Carefully dispense 1 bead into each testing well, avoiding splashes.
7. Cover the reaction tray using an adhesive plate cover. Gently tap the tray to remove trapped air bubbles and incubate the plate at room temperature (15-30⁰C) for 16-20 hours.
8. Remove the cover from the reaction tray and discard. Wash each bead.
9. Add 200 μ L Antibody Solution to all testing wells. Cover the reaction tray using a new adhesive plate cover. Gently tap the tray to remove trapped air bubbles and incubate the plate at 40 \pm 2⁰C for 4 hours \pm 10 minutes.
10. Remove the cover from the reaction tray and discard. Wash each bead.
11. Add 200 μ L of Conjugate Solution to all testing wells. Cover the plate using a new adhesive plate cover. Gently tap the tray to remove trapped air bubbles and incubate the plate at 40 \pm 2⁰C for 1 hour \pm 10 minutes.
12. Remove the cover from the reaction tray and discard. Wash each bead.
13. Immediately transfer the beads to a properly identified box of assay tubes. Add 300 μ L of OPD substrate solution into 2 empty tubes (substrate blanks) and then into all tubes containing a bead. Cover the tubes to prevent exposure to intense light. Incubate at room temperature (15-30⁰C) for 30 \pm 2 minutes.
14. Add 1 mL of Stop Solution to all tubes.

15. Blank spectrophotometer with one of the substrate blank tubes at 492 nm. Read the absorbance of each tube at 492 nm within 2 hours of the addition of Stop Solution.

VI. CALCULATIONS

The HIV-1 p24 antigen concentrations may be generated from a virology data-management software program developed for the Division of AIDS (DAIDS) to ensure data integrity of both QA and test specimens. A weighted linear least squares method using the VQA standard concentrations is used to estimate HIV-1 p24 antigen concentration.

VII. QUALITY CONTROL

The absorbances obtained from the spectrophotometer may be transferred into the virology data-management software program. The software program incorporates two QC check programs, Cum Sum and Levy Jennings. These two programs review the absorbance of the VQA standards, and the VQA media QC and compare them to established standard deviation ranges. These ranges are determined by the testing laboratory and is reflective of values unique to each laboratory. The software will flag values that fall outside of the laboratory's standard deviation range. The technician must determine the significance of the out of range QC and resolve the situation.

VIII. PROCEDURAL NOTES

When dispensing beads, remove cap from bead bottle, attach Bead Dispenser and dispense beads into wells of the reaction tray. Beads may also be dispensed using plastic forceps.

Do not splash liquid when tapping trays.

When transferring beads from wells to assay tubes, align inverted rack of orientated tubes over the reaction tray. Take care that well A1 aligns with tube A1! Press the tubes tightly over the wells and invert tray and tubes together so the beads fall into corresponding tubes. Blot excess water from the top of the tube rack.

Dispense acid in same tube sequence as OPD Substrate Solution.

IX. REFERENCES

Abbott HIV-1 Antigen Assay package insert and all references within.